

CLAIMS

1. Isopenicillin N synthase (IPNS) in the form of: a complex with  
5 Fe and its substrate, said complex having a structure substantially  
designated by the X-ray co-ordinates in Table 3.
2. The IPNS complex of claim 1, wherein the substrate is  
L- $\delta$ - $\alpha$ -aminoadipoyl-L-cysteinyl-D-valine (ACV).
3. The IPNS complex of claim 1 wherein the substrate is an  
10 analogue of ACV selected from AC glycine, Ac aminobutyrate, AC alanine  
and AC propargylglycine.
4. Use of the three dimensional structure of a first enzyme  
selected from IPNS, DAOCS, DACS, DAOC/DACS and other related  
enzymes of the penicillin and cephalosporin biosynthesis pathway, for the  
15 modification of a second enzyme selected from IPNS, DAOCS, DACS,  
DAOC/DACS and other related enzymes of the penicillin and  
cephalosporin biosynthesis pathway.
5. Use as claimed in claim 4, wherein the second enzyme is  
modified: to accept unnatural substrates for the preparation of antibacterial  
20 materials or intermediate for the production of pharmaceutical products; or  
to produce unnatural products or improve the production of natural  
products.
6. An enzyme having significant (as herein defined) sequence  
similarity to IPNS, wherein at least one of the following amino acid residues  
25 is modified:  
N287; R87; A88; Y189; S183; Y91; F285; Q330; T331;  
V185; L106; C104; V217; L324; L317; I325; L321; S210.
7. An enzyme having significant (as herein defined) sequence  
similarity to IPNS, wherein at least one of the following amino acid residues  
30 is modified:

V272; L231; L223; P283; T221; F211, F285; Q330;  
I187; V185; Y189; R279; S281; N230; Q225; N252; S210.

8. A gene which codes for the enzyme of claim 6 or claim 7.

9. A micro-organism containing the gene of claim 8 and which is  
5 capable of expressing the gene under fermentation conditions.

10. Use of the micro-organism of claim 9 for making a bicyclic  
 $\beta$ -lactam of the penicillin or cephalosporin (including cephams) families.

11. Use of the enzyme of claim 6 or claim 7 for the preparation *in*  
*vitro* of a bicyclic  $\beta$ -lactam of the penicillin or cephalosporin families.

10 12. In a method for the preparation of an enzyme, selected from  
IPNS, DAOCS, DACS, DAOC/DACS and sequence-related enzymes, in  
crystalline form for X-ray diffraction studies, the improvement which  
consists in maintaining the enzyme under anaerobic conditions with  
dioxxygen substantially absent.

15 13. A method which comprises using the three dimensional  
structure of a first enzyme selected from IPNS, DAOCS, DACS,  
DAOC/DACS and other related enzymes of the penicillin and  
cephalosporin biosynthesis pathway, for determining or predicting the  
structure of a second enzyme which is structurally related to the first  
20 enzyme but is not active in the penicillin or cephalosporin biosynthesis  
pathway, and using the structural information so obtained for modifying the  
second enzyme or for designing an inhibitor for the second enzyme.

14. Use of the enzyme of claim 6 or claim 7 to convert a  
dipeptide to a 6- aminopenicillin or other bicyclic  $\beta$ -lactam.

25 15. Use as claimed in claim 14, wherein the dipeptide has been  
produced by use of a peptide synthetase enzyme such as  
L- $\delta$ - $\alpha$ -amino adipoyl-L-cysteinyl-D-valine (ACV) synthetase optionally  
modified to optimise dipeptide production.